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May 23, 2003

3M

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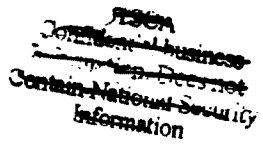
Document Processing Center (7407)
Office of Pollution, Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N. W.
Washington, DC 20460
Attention: Section 8(e) Coordinator



Re: **TSCA Section 8(e) Submissions**

Dear Sir/Madam:

3M Company ("3M") requests that EPA place the attached studies in the TSCA Section 8(e) docket. We have included an index for these studies identifying the study title, test substance and CAS number. A CBI version of this index and the studies also is being submitted today pursuant to EPA procedures.

3M has concluded that data in these studies may not be, strictly speaking, "corroborative" of previously reported or published information as defined in EPA's reporting guidance or otherwise potentially may warrant 8(e) submission based on EPA's reporting guidance.

3M appreciates EPA's attention to this matter. Please contact the undersigned if you have any questions or require further information regarding this submission.

Very truly yours,

Dr. Katherine E. Reed (9#)

Dr. Katherine E. Reed, Ph.D
Executive Director
3M Environmental Technology
And Safety Services
(651) 778-4331
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8EHQ-03-15366

8EHQ0000161

Contains No CBI

SUBMISSION BY 3M COMPANY ON MAY 23, 2003

1.	Exploratory 28-Day Oral Toxicity Study with T-7250, T-7251, T-7252, T-7253, T-7254, and T-7255 by Daily Gavage in the Rat Followed by a 14/28-Day Recovery Period (NOTOX Project 264656)	Separate studies for each chemical: [CBI removed]; Hexanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,6 - Tridecafluoro-n-(2-Hydroxyethyl)-N-Methyl - 100%; 1-Butanesulfonamide, 1,1,2,2,3,3,4,4,4-Nonafluoro-N-n(2-Hydroxyethyl)-N-Methyl - 100%	[CBI removed]; 68555-75-9; 34454-97-2
2.	Exploratory 28-Day Oral Toxicity Study with T-7125, T-7126, T-7127, T-7128, and T-7129 by Daily Gavage in the Rat Followed by a 14/28-Day Recovery Period (NOTOX Project 256679)	Cyclohexanesulfonic acid, decafluoro(pentafluormethyl)-, potassium salt (CAS No. 67584-42-3) - 66-70%; Cyclohexanesulfonic acid, decafluoro(trifuloromethyl)-, potassium salt (CAS No. 68156-07-0) - 18-22%; Cyclohexanesulfonic acid, nonafluorobis(trifluoromethyl)-, potassium salt (CAS No. 68156-01-4) - 9-13%; Cyclohexanesulfonic acid, undecafluoro-, potassium salt (CAS No. 3107-18-4) - 1-3%	67584-42-3; 68156-07-0; 68156-01-4; 3107-18-4
3.	Subchronic 90-Day Oral Toxicity with T-6524 by Daily Gavage in the Rat Followed by a 28-Day Recovery Period	65% Sulfonamides, C4-8-alkane, perfluoro, N-(3-(dimethyloxidoamino)propyl), potassium CAS#179005-06-2; 20% Amine oxide C8F17SO2NH(->O)CH2CH2CH2N(CH3)2; 15% C3-C7 K-salts of amine oxides CNF2N+1SO2N-)(+K)(->O)CH2CH2CH2N(CH3)2	179005-06-2
4.	A Study for Effects on Embryofoetal Development of the Rat (Inhalation Administration)	[CBI removed]	[CBI removed]
5.	Evaluation of the Ability of T-5870 to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes (with Independent Repeat)	2-ethoxy ethyl acrylate	106-74-1
6.	Chromosomal Aberration Test of T-6695 Using Cultured Mammalian Cells	[CBI removed]	[CBI removed]
7.	Acute Oral Toxicity Study in Rats (Exp. No. 920584) (Test Article: Intermedio 1249)	2-methyl-2-butanone-(4-sulfonamidophenyl)-hydrazone; Molecular Formula: C11H17N3O2S	Unknown
8.	Acute Oral Toxicity Study in Rats (Exp. No. 930321) (Test Article: 501149)	3H-pyrazol-3-0ne, 2-(4-aminophenyl), 4-dihydro-5-(1-pyrrolidinyl)	30707-77-8
9.	Skin Corrosivity Study of T-5799 in Rabbits (DOT/UN Regulations)	1-Octanesulfonyl Fluoride - 87.5%, Other Alkyl Sulfonyl Fluorides and Acidic Impurities - 11%, Water - 5.4%, Octanesulfonyl Chloride - 1.4%	40630-63-5; Unknown; 7732-18-5; 7795-95-1
10.	Skin Corrosivity Study of T-5800 in Rabbits (DOT/UN Regulations)	1-Octanesulfonyl Fluoride - 87.5%, Other Alkyl Sulfonyl Fluorides and Acidic Impurities - 11%, Water - 5.4%, Octanesulfonyl Chloride - 1.4%.	40630-63-5; Unknown; 7732-18-5; 7795-95-1
11.	Primary Dermal Irritation/Corrosion Study of T-5635 in Rabbits (OECD Guidelines)	[CBI removed]	[CBI removed]
12.	Primary Dermal Irritation/Corrosion Study of T-5897 in Rabbits (OECD Guidelines)	Isophthaloylbis (2-methylarziridine) - 97%, Toluene - 2%, Xylene - 0.5%.	7652-64-4; 108-88-3; 1330-20-7
13.	Skin Corrosivity Study of T-7030.1 in Rabbits (with Protocol TP4206 attached)	[CBI removed]	[CBI removed]
14.	Dermal Sensitization Study of T-5474 in Guinea Pigs - Maximization Test (EPA Guidelines)	Water (CAS No. 7732-18-5) - 68.4%; Dodecylbenzenesulfonic Acid (CAS No. 27176-87-0) - 17.5%; Polymethacrylate (CAS No. 25087-26-7) - 11.76%; Sodium Hydroxide (CAS No. 1310-73-2) - 2.3%; Unknown - 0.040%	7732-18-5; 27176-87-0; 25087-26-7; 1310-73-2

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15	Dermal Sensitization Study of T-5894 in Guinea Pigs - Maximization Test (EC Guidelines) (with Protocol TP6164E attached)	[CBI removed]	[CBI removed]
16.	Dermal Sensitization Study of T-6006 in Guinea Pigs - Closed Patch Technique (EPA Guidelines)	Dimethyltetradecylamine Oxide - 55%, Oleamidopropyldimethylamine - 18%, 1-Methoxy -2-Propanol – 5%, Citronellol – 5%, Polyethylene Glycol - < 3%, Alpha - (Carboxymethyl) - Omega - (Dodecyloxyl) Poly (Oxyethylene) Sodium Salt - ~3%, Trialkyl Amine Oxide - 2%, Isopropyl Alcohol - 2%, Fragrance Sozio SZ 5467 - 2%, Water - 1%, Acetic Acid – 1%, Miscellaneous ingredients at less than 1%	3332-27-2; 109-28-4; 107-98-2; 106-22-9; 25322-68-3; 33939-64-9; 7128-91-8; 67-63-0; Unknown; 7732-18-5; 64-19-7
17.	Dermal Sensitization Study of T-7280 in Guinea Pigs - Closed Patch Technique (with Protocol TP2008 attached)	[CBI removed]	[CBI removed]
18.	Acute Oral Toxicity Study of T-6735 in Rats (OECD Guidelines) (with Protocol TP2069 attached)	4,6-dibromo-2-isopropyl phenol	Unknown
19.	Acute Toxicity to Daphnia Magna	[CBI removed]	[CBI removed]
20.	Evaluation of the Mutagenic Activity of T-5870 in an In Vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells (with Independent Repeat)	2-ethoxy ethyl acrylate	106-74-1
21.	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 920364) (Test Article: 586442-50055)	HP=Benzothiazolium (9CI); SB=3-ethyl-2-((3-(3-(3-ethyl-2(3H)-benzothiazolylidene)-1-propenyl)-5,5-dimethyl-2-cyclohexen-1-ylidene)methyl)-6-methoxy-5-methyl-; NM=Iodide; Molecular Formula: C32H37N2OS2.I	87699-86-3
22.	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 940151) (Test Article: 580066)	Thiazolium, 3-ethyl-2-[3-(3-ethyl-2-thiazolidinylidene)-1-propenyl]-4,5-dihydro-,iodide; Molecular Formula: C13H21N2S2.I	3065-71-2
23.	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 930529) (Test Article: 1268)	3-ethoxy-carbonyl-methyl-4-etoxy-methylidene-rhodanine; Molecular Formula: C10H13NO4S2	Unknown
24	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 920582) (Test Article: 1248)	C6H10ClN3O2S	Unknown
25.	One Generation Reproduction Study of PFOS - Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats	Perfluorooctane Sulfonic Acid Potassium Salt	2795-39-3
26	Augmented acute (4-hour) inhalation toxicity study with T-6905 in rats	2% solids of fluorochemical fatty acid ester in water	306974-63-0

REPORT

EVALUATION OF THE ABILITY OF

T-5870.2

TO INDUCE CHROMOSOME ABERRATIONS IN CULTURED

PERIPHERAL HUMAN LYMPHOCYTES

(WITH INDEPENDENT REPEAT)

NOTOX Project 115976
NOTOX Substance 38205

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

With the following exception:

Stability of the test substance in the vehicle was unknown.

Study Director Ing. E.J. van de Waart

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Date: 05/07/1994

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands.

Study procedures were subject to periodic inspections and general non study specific processes were also inspected at periodic intervals.

This report was audited by the NOTOX Quality Assurance Unit and the methods and results accurately reflect the raw data.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
10-02-1994 23-03-1994 13-04-1994 09-06-1994	10-02-1994 24-03-1994 14-04-1994 09-06-1994

Quality Assurance Manager

C.J. Mitchell B.Sc.

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Date: July 06, 1994

REPORT APPROVAL

STUDY DIRECTOR: Ing. E.J. van de Waart

.....

Date: 05/07/1994

MANAGEMENT: Dr. I.C. Enninga
Technical Director

i.a.

Ir. J.M. Loodinaals

.....

Date: 5 July, 1994

PREFACE

Sponsor	3M Belgium - Chemical EBC Canadastraat 11 B-2070 ZWIJNDRECHT Belgium
Study Monitor	Mr. R.H. Cox
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ing. E.J. van de Waart
Technical Coordinator	A.M.C. Bertens
Study Plan	Start : March 02, 1994 Completed : May 08, 1994

TEST SUBSTANCE

Identification	T-5870
Description	Clear liquid
Batch	162
Purity	100%
Specific Gravity	0.985
Instructions for test substance storage	At room temperature (< 25°C) in the dark
Stability under storage conditions	Stable
Expiry date	May 01, 1994
Stable for at least 4 hours in vehicle	Water : not indicated Dimethyl sulphoxide: not indicated

VEHICLE

The test substance was dissolved in dimethylsulphoxide of spectroscopic quality (Merck). Test substance concentrations were prepared directly prior to use. The final concentration of the solvent in the culture medium amounted to 0.9 % (v/v). The pH and the osmolarity of the culture medium containing 3330 µg/ml test substance were recorded.

GUIDELINES

The study procedures described in this report were based on the following guidelines:

- Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Guideline no. 473: "Genetic Toxicology: In Vitro Mammalian Cytogenetic Test", (adopted May 26, 1983).
- European Economic Community (EEC), Directive 92/69/EEC. Annex V of the EEC Directive 67/548/EEC, Part B: Methods for the Determination of Toxicity; B.10: "Other Effects-Mutagenicity: In Vitro Mammalian Cytogenetic Test". EEC Publication no. L383 (adopted December, 1992).

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test article reference sample, all specimens and raw data.

OBJECTIVE

Purpose of the study

The objective of this study was to evaluate the test substance for its ability to induce structural chromosome aberrations in cultured human lymphocytes, either in the presence or absence of a metabolic system (S9-mix).

Justification and rationale of the test system

Stimulated cultured human lymphocytes were used because they are sensitive indicators of clastogenic activity of a broad range of chemical classes. In combination with a mammalian metabolizing system (S9-mix) also indirect chemical mutagens, i.e. those requiring metabolic transformation into reactive intermediates, could be tested for clastogenic effects in vitro.
Following treatment, cell division was arrested in the metaphase stage of the cell cycle by addition of the spindle poison colchicine. Structural chromosome changes such as breaks, gaps, minutes, dicentrics and exchange figures were examined microscopically in cultures treated with the test substance and the results were compared with those of the control (vehicle-treated) cultures.
Chromosome aberrations were generally evaluated in the first post-treatment mitosis. The appearance of the first post-treatment mitosis could be considerably delayed, due to toxic insult of the cells. Therefore, cells were harvested at 24 h and 48 h after beginning of treatment to cover the interval in which maximum aberration frequency was expected.
A test article which induced a positive response in this assay was presumed to be a potential mammalian cell clastogenic agent.

MATERIALS AND METHODS

TEST SYSTEM

Test System	Cultured peripheral human lymphocytes
Rationale	Recognized by the international guidelines as the recommended test system (e.g. EPA, OECD, EEC).
Source	Healthy adult male volunteers: pilot study : age 27, AGT = 14.4 h (Dec.'93) experiment 1: age 27, AGT = 15.5 h (Mar.'94) experiment 2: age 34, AGT = 16.6 h (Dec.'93) (AGT = Average Generation Time)

CELL CULTURE

Blood samples	Blood samples were taken from a healthy adult male volunteer by venapuncture using the Venoject multiple sample blood collecting system with a suitable size sterile vessel containing sodium heparin. The blood samples were stored at a temperature between 4 and 25°C. Within 4 h after withdrawal lymphocyte cultures were started.
F10 complete culture medium	F10 complete culture medium consisted of Ham's F10 medium without thymidine and hypoxanthine (Gibco), supplemented with 20% heat-inactivated (56°C; 30 min) foetal calf serum (Gibco), L-glutamine (2 mM), penicillin/streptomycin (50 U/ml and 50 µg/ml respectively), sodium bicarbonate (2 g/l) and 30 U/ml heparin.
Cell culture conditions	Whole blood was cultured in F10 complete culture medium with Phytohaemagglutinin (Murex). Per culture (5 ml F10 complete culture medium and 0.4 ml whole blood) 0.1 ml (9 mg/ml) Phytohaemagglutinin was added.
Environmental conditions	All incubations were carried out in a humid atmosphere (80-95%) containing 5% CO_2 in air in the dark at 37°C. The temperature, humidity and CO_2-percentage were monitored during the experiment.

REFERENCE SUBSTANCES

Negative control:
The vehicle of the test article.

Positive controls:

Without metabolic activation (-S9-mix):
Mitomycin C (MMC-C; CAS no. 50-07-7, Sigma, U.S.A.) was used as a direct acting mutagen at a final concentration of 0.2 µg/ml (solvent: HBSS) for a 24 h treatment period and 0.1 µg/ml (solvent: HBSS) for a 48 h treatment period.

With metabolic activation (+S9-mix):
Cyclophosphamide (CP; CAS no. 50-18-0. Endoxan-Asta, Asta-Werke, F.R.G.) was used as an indirect acting mutagen, requiring metabolic activation, at a final concentration of 15 µg/ml (solvent: HBSS) for a 3 h treatment period (24 h fixation period).

Solvent for reference substances
HBSS = Hank's Balanced Salt Solution without calcium and magnesium.

METABOLIC ACTIVATION SYSTEM

Preparation of S9-homogenate*:

Rat liver microsomal enzymes were routinely prepared from adult male Wistar or Sprague Dawley rats, which were obtained from BRL, Switzerland.

The animals were housed at NOTOX in a special room under standard laboratory conditions, as described in the SOP's. The rats were injected intraperitoneally with a solution (20% w/v) of Aroclor 1254 (500 mg/kg body weight) in corn oil. Five days later, they were killed by decapitation; (they were denied access to food for at least 12 hours preceding sacrifice). The livers of the rats were removed aseptically, and washed in cold (0°C) sterile 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1 mM Na_2-EDTA. Subsequently the livers were minced in a blender and homogenized in 3 volumes of phosphate buffer with a Potter homogenizer. The homogenate was centrifuged for 15 min at 9000 g. The supernatant (S9) was transferred into sterile ampules, which were stored in liquid nitrogen (-196°C).

*) Ames, B.N., McCann, J. and Yamasaki, E., 1975, Methods for detecting carcinogens and mutagens with the Salmonella/Mammalian microsome mutagenicity test, Mutation Res., 31, 347-364.

Preparation of S9-mix:

S9-mix was prepared immediately before use and kept on ice during the test. S9-mix contained per ml: 1.02 mg $MgCl_2.6H_2O$; 2.46 mg KCl; 1.7 mg glucose-6-phosphate; 3.4 mg NADP; 4 µmol HEPES and 0.5 ml S9. The above solutions were mixed and filter (0.22 µm)-sterilized (apart from the S9-fraction, which was added after filter-sterilization of the S9-mix components). Metabolic activation was achieved by adding 0.2 ml liver S9-mix to each cell suspension.

EXPERIMENTAL PROCEDURE

Cytogenetic test

The test was carried out with minor modifications as described by Evans (1984)*.

The test substance was tested both with and without S9-mix in duplicate in two independent experiments.
Lymphocyte cultures (0.4 ml blood of a healthy male donor in 5 ml medium) were cultured for 48 h and thereafter exposed in duplicate to selected doses of the test substance for 24 h and 48 h without S9-mix or for 3 h with S9-mix. An appropriate range of dose levels was chosen to determine the concentrations which caused inhibition of the mitotic index. In case the test compound was difficult to dissolve in aqueous solutions the highest concentration was determined by the solubility in the culture medium. Concentrations exceeding 5 mg/ml were not tested. After 3 h treatment, the cells exposed to the test substance in the presence of S9-mix were rinsed once with 5 ml of HBSS and incubated in 5 ml growth medium for another 20-22 h (first fixation period) or for 44-46 h (second fixation period). The cells which were treated for 24 h and 48 h in the absence of S9-mix were not rinsed after treatment and were fixed immediately after 24 h and 48 h. During the last 3 h of the culture period, cell division was arrested by addition of the spindle inhibitor colchicine (0.5 µg/ml medium). Thereafter the cell cultures were centrifuged for 5 min at 1300 rpm (150 g) and the supernatant was removed. Cells in the remaining cell pellet were swollen by a 5 min treatment with hypotonic 0.56% potassium chloride solution at 37°C. After hypotonic treatment, cells were fixed with 3 changes of methanol: acetic acid fixative (3:1 v/v).
For the independent repeat the 24 h fixation period was needed only.

*) Evans, H.J., 1984, Human Peripheral Blood Lymphocytes for the Analysis of Chromosome Aberrations in Mutagen Tests. In: Handbook of Mutagenicity Test Procedures, B.J. Kilbey, M. Legator, W. Nichols and C. Ramel eds, 405-427, Elsevier Science Publishers B.V., Amsterdam.

Preparation of slides

Fixed cells were dropped onto previously cleaned (24 hours immersed in a 1:1 mixture of 96% ethanol/ether and cleaned with a tissue) and marked (with the NOTOX study identification number and group number) slides. Two slides were prepared per culture. Slides were allowed to dry and thereafter stained for 10-30 min with 5% (v/v) Giemsa solution in tap water. Thereafter slides were rinsed in tap-water and allowed to dry. The dry slides were cleared by dipping them in xylene before they were embedded in DePeX and mounted with a coverslip.

Mitotic index/dose selection

The mitotic index of each culture was determined by counting the number of metaphases per 1000 cells. For the first fixation time (24 h harvest) chromosomes of metaphase spreads were analysed of those cultures with an inhibition of the mitotic index of about 50-20% (if present) whereas the mitotic index of the lowest dose level was approximately the same as the solvent control. Also cultures treated with an intermediate dose were examined for chromosome aberrations. For the second fixation time (48 h harvest) one appropriate dose level was selected for scoring of chromosome aberrations.
In order to obtain the appropriate concentration range for the chromosome aberration test a pilot experiment was performed. Experimental conditions were identical to those in the chromosome aberration test, except that one culture per concentration was used and with the omission of the 48 h fixation period in the presence of S9-mix.

Analysis of slides for chromosome aberrations

For control of bias, all slides were randomly coded before examination of chromosome aberrations and scored. An adhesive label with NOTOX study identification number and code was stuck over the marked slide. At least 100 metaphase chromosome spreads per culture were examined by light microscopy for chromosome aberrations. Only metaphases containing 46 chromosomes were analysed. The number of cells with aberrations and the number of aberrations were calculated.

ACCEPTABILITY OF ASSAY

A chromosome aberration test was considered acceptable if it met the following criteria:
a) The numbers of chromosome aberrations found in the solvent control cultures should reasonably fall within the laboratory historical control data range.
b) The positive control substances should produce a statistically significant (Chi-square test, $P < 0.05$) increase in the number of cells with chromosome aberrations.

DATA EVALUATION AND STATISTICAL PROCEDURES

A test substance was considered positive (clastogenic) in the chromosome aberration test if:
a) It induced a dose-related statistically significant (Chi-square test, $P < 0.05$) increase in the number of cells with chromosome aberrations.
b) A statistically significant increase in the frequency of aberrations was observed in the absence of a clear dose-response relationship, but the results were reproducible in an independently repeated experiment.

A test substance was considered negative (not clastogenic) in the chromosome aberration test if:
a) None of the tested concentrations induced a statistically significant (Chi-square test, $P < 0.05$) increase in the number of cells with chromosome aberrations.

The preceding criteria were not absolute and other modifying factors might enter into the final evaluation decision.

The incidence of aberrant cells (cells with one or more chromosome aberrations, inclusive or exclusive gaps) for each treatment group was compared to that of the solvent control using Chi-square statistics:

$$X^2 = \frac{(N-1) \times (ad-bc)^2}{(a+b)\ (c+d)\ (a+c)\ (b+d)}$$

where b = the total number of aberrant cells in the control cultures.
d = the total number of nonaberrant cells in the control cultures.
n_0 = the total number of cells scored in the control cultures.
a = the total number of aberrant cells in treated cultures to be compared with the control.
c = the total number of nonaberrant cells in treated cultures to be compared with the control.
n_1 = the total number of cells scored in the treated cultures.
N = sum of n_0 and n_1

$$\text{If } P \left| \overline{X^2} > \frac{(N-1) \times (ad-bc)^2}{(a+b)\ (c+d)\ (a+c)\ (b+d)} \right| \quad \text{(two-tailed)}$$

is small ($P < 0.05$) the hypothesis that the incidence of cells with chromosome aberrations is the same for both the treated and the solvent control group is rejected and the number of aberrant cells in the test group is considered to be significantly different from the control group at the 95% confidence level.

RESULTS

DOSE SELECTION

In a preliminary study blood cultures were treated with 33, 100, 333, 1000 and 3330 µg T-5870 per ml culture medium with and without S9-mix. Higher concentrations were not tested because of toxicity in NOTOX project 115987. The pH and osmolarity of a concentration of 3330 µg/ml were 8.12 and 411 mOsm/kg respectively (compared to 8.06 and 444 mOsm/kg in the solvent control).

TABLE 1 MITOTIC INDEX OF DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870
PILOT STUDY

Test substance concentration (µg/ml)	Number of metaphases per 1000 cells	
	Absolute	Percentage of control
	Without metabolic activation (-S9-mix)	
24 h fixation period		
Control[a)]	27	100
33	13	48
100	0	0
333	0	0
1000	0	0
3330	0	0
48 h fixation period		
Control[a)]	28	100
33	21	75
100	0	0
333	0	0
1000	0	0
3330	0	0
	With metabolic activation (+S9-mix)	
24 h fixation period		
Control[a)]	84	100
33	66	79
100	54	64
333	61	73
1000	0	0
3330	0	0

a) DMSO

Based on the results of this pilot study the following dose levels were selected to determine a concentration which caused the appropriate inhibition of the mitotic index:

Experiment 1 + 2

Without S9-mix : 3, 10, 17.8, 33 and 56 µg/ml culture medium
(24 h fixation period)
17.8, 33 and 56 µg/ml (48 h fixation period)
With S9-mix : 33, 100, 333, 422, 562 and 750 µg/ml culture medium
(24 h fixation period)
333, 422, 562 and 750 µg/ml (48 h fixation period)

Tables 2 and 3 show the mitotic index of cultures (from blood of a healthy male donor) treated with various test substance concentrations or with the positive or negative control substances.

Based on these observations the following doses were selected for scoring of chromosome aberrations:

Experiment 1

Without S9-mix : 3, 10 and 17.8 µg/ml culture medium
(24 h fixation period)
33 µg/ml (48 h fixation period)
With S9-mix : 33, 100 and 422 µg/ml culture medium
(24 h fixation period)
422 µg/ml (48 h fixation period).

Experiment 2

Without S9-mix : 10, 17.8 and 33 µg/ml culture medium
(24 h fixation period)
With S9-mix : 33, 100 and 333 µg/ml culture medium
(24 h fixation period)

CYTOGENETIC TEST

The ability of T-5870 to induce chromosome aberrations in human peripheral lymphocytes was investigated. The test was carried out in duplicate in two independent experiments. The results of duplicate cultures are indicated by A and B. The scores for the numbers of aberrant cells (inclusive and exclusive gaps) and the numbers of the various types of chromosome aberrations at the various concentrations of the test substance are presented in Tables 4-9. The criteria according to which the aberrations were classified are outlined in Appendix 1.

Both in the presence and absence of S9-mix the test substance did induce a statistically and biologically significant increase in the number of cells with chromosome aberrations.

The number of cells with chromosome aberrations found in the solvent control cultures were within the laboratory historical control data range {i.e. 1.0 ± 1.1 (mean ± standard deviation) aberrant cells per 100 metaphases (without S9-mix; gaps excluded) and 0.9 ± 2.4 aberrant cells per 100 metaphases (with S9-mix; gaps excluded)}. The positive control chemicals (MMC-C and CP) both produced statistically significant increases in the frequency of aberrant cells. It was therefore concluded that the test conditions were optimal and that the metabolic activation system (S9-mix) functioned properly.

Finally, it is concluded that this test should be considered valid and that T-5870 is clastogenic under the experimental conditions of this test.

TABLE 2 MITOTIC INDEX OF DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870
Experiment 1

Test substance concentration (µg/ml)	Number of metaphases per 1000 cells b) Absolute	Percentage of control
	Without metabolic activation (-S9-mix)	
24 h fixation period		
Control[a)]	35 - 50	100
3	42 - 40	96
10	30 - 32	73
17.8	30 - 25	65
33	7 - 10	20
56	0 - 0	0
MMC-C; 0.2 µg/ml	18 - 23	48
48 h fixation period		
Control[a)]	42 - 38	100
17.8	38 - 39	96
33	21 - 20	51
56	0 - 0	0
MMC-C; 0.1 µg/ml	27 - 26	66
	With metabolic activation (+S9-mix)	
24 h fixation period		
Control[a)]	66 - 82	100
33	50 - 72	82
100	44 - 44	59
333	42 - 46	59
422	40 - 34	50
562	29 - 24	36
750	1 - 3	3
CP; 15 µg/ml	27 - 23	34
48 h fixation period		
Control[a)]	72 - 67	100
333	45 - 41	62
422	43 - 38	58
562	24 - 27	37
750	11 - 10	15

a) DMSO
b) duplicate cultures

TABLE 3 MITOTIC INDEX OF DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870
Experiment 2

Test substance concentration (µg/ml)	Number of metaphases per 1000 cells b) Absolute	Percentage of control
	Without metabolic activation (-S9-mix)	
24 h fixation period		
Control[a)]	29 - 28	100
3	23 - 24	82
10	27 - 23	88
17.8	29 - 18	82
33	23 - 21	77
56	3 - 3	11
MMC-C; 0.2 µg/ml	12 - 16	49
	With metabolic activation (+S9-mix)	
24 h fixation period		
Control[a)]	40 - 49	100
33	39 - 34	82
100	37 - 40	87
333	30 - 27	64
422	16 - 15	35
562	19 - 17	40
750	3 - 3	7
CP; 15 µg/ml	21 - 17	43

a) DMSO
b) duplicate cultures

TABLE 4 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870 (Without S9-mix)[a)]
24 h fixation period
Experiment 1

Conc µg/ml	DMSO (0.9% v/v)			3 µg/ml			10 µg/ml			17.8 µg/ml			MMC-C 0.2 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	0	1	1	5	4	9*	4	3	7*	7	3	10**	42	44	86***
No. of cells with aberrations (- gaps)	0	1	1	5	2	7*	3	2	5	5	2	7*	36	41	77***
g'					2		1	1		2	1		9	7	
g"													1		
b'				3	1		2			4	1		28	27	
b"		1		2	1		1	2		1	1		11	17	
m'															
m"					1										
exch.													1	6	
dic															
d'															
misc.															
total aberr (+ gaps)	0	1		5	5		4	3		7	3		50	57	
total aberr (- gaps)	0	1		5	3		3	2		5	2		40	50	

a) Abbreviations used for various types of aberrations are listed in appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.
*) Significantly different from control group (Chi-square test),
* $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 5 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870 (With S9-mix)[a)]
24 h fixation period
Experiment 1

Conc µg/ml	DMSO (0.9% v/v)			33 µg/ml			100 µg/ml			422 µg/ml			CP 15 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	1	2	3	2	2	4	5	1	6	13	3	16**	40	36	76***
No. of cells with aberrations (- gaps)	0	1	1	1	1	2	4	0	4	12	2	14***	33	27	60***
g'	1	1		1	1		1	1		2	1		9	15	
g"													1	1	
b'				1			1			14	1		22	17	
b"		1			1		3			7	1		15	9	
m'														1	
m"										1					
exch.										2				3	
dic															
d'															
misc.										ma					
total aberr (+ gaps)	1	2		2	2		5	1		36	3		47	46	
total aberr (- gaps)	0	1		1	1		4	0		34	2		37	30	

a) Abbreviations used for various types of aberrations are listed in appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.
misc.= (miscellaneous) aberrations not belonging to the ones mentioned above.
*) Significantly different from control group (Chi-square test),
* $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 6 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870 (Without S9-mix)[a)]
48 h fixation period
Experiment 1

Conc µg/ml	DMSO (0.9% v/v)			33 µg/ml			MMC-C 0.1 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	50	150
No. of cells with aberrations (+ gaps)	1	1	2	9	12	21***	45	26	71***
No. of cells with aberrations (- gaps)	1	1	2	7	12	19***	43	26	69***
g'				3	3		7	4	
g"									
b'	1	1		3	15		43	18	
b"				4	5		20	13	
m'									
m"							1		
exch.							1	1	
dic									
d'									
misc				ma	endo 2 ma				
total aberr (+ gaps)	1	1		20	43		72	36	
total aberr (- gaps)	1	1		17	40		65	32	

c) Abbreviations used for various types of aberrations are listed in appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.
misc.= (miscellaneous) aberrations not belonging to the ones mentioned above.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 7 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870 (With S9-mix)[a)]
48 h fixation period
Experiment 1

Conc µg/ml	DMSO (0.9% v/v)			422 µg/ml		
Culture	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	3	1	4	4	1	5
No. of cells with aberrations (- gaps)	1	0	1	3	0	3
g'	2	1		1	1	
g"						
b'	1			1		
b"				1		
m'						
m"						
exch.						
dic						
d'						
misc.				ma		
total aberr (+ gaps)	3	1		13	1	
total aberr (- gaps)	1	0		12	0	

a) Abbreviations used for various types of aberrations are listed in appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.
misc.= (miscellaneous) aberrations not belonging to the ones mentioned above.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 8 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870 (Without S9-mix)[a)]
24 h fixation period
Experiment 2

Conc µg/ml	DMSO (0.9% v/v)			10 µg/ml			17.8 µg/ml			33 µg/ml			MMC-C 0.2 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	50	50	100
No. of cells with aberrations (+ gaps)	2	2	4	2	1	3	4	13	17**	8	15	23***	29	27	56***
No. of cells with aberrations (- gaps)	1	0	1	1	1	2	4	12	16***	7	15	22***	29	27	56***
g'	1	2		1				2		1	1		3	3	
g"															
b'				1	1		3	10		4	11		17	16	
b"	1						1	3		3	5		10	9	
m'															
m"															
exch.										1	1		6	7	
dic															
d'															
misc.													ma		
total aberr (+ gaps)	2	2		2	1		4	15		9	18		46	35	
total aberr (- gaps)	1	0		1	1		4	13		8	17		43	32	

a) Abbreviations used for various types of aberrations are listed in appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.
*) Significantly different from control group (Chi-square test),
* $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 9 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH VARIOUS CONCENTRATIONS OF T-5870 (With S9-mix)[a)]
24 h fixation period
Experiment 2

Conc µg/ml	DMSO (0.9% v/v)			33 µg/ml			100 µg/ml			333 µg/ml			CP 15 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	50	50	100
No. of cells with aberrations (+ gaps)	2	2	4	2	1	3	2	1	3	4	7	11	27	25	*** 52
No. of cells with aberrations (- gaps)	0	1	1	1	0	1	1	1	2	4	6	** 10	27	25	*** 52
g'	2	1		1	1		1				1		4	1	
g"															
b'		1					1	1		2	1		19	17	
b"		1		1							2		10	8	
m'															
m"															
exch.										2	2		2	2	
dic															
d'															
misc.					endo		poly				3poly ma				
total aberr (+ gaps)	2	3		2	1		2	1		4	16		35	28	
total aberr (- gaps)	0	2		1	0		1	1		4	15		31	27	

a) Abbreviations used for various types of aberrations are listed in appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.
misc.= (miscellaneous) aberrations not belonging to the ones mentioned above.
*) Significantly different from control group (Chi-square test),
* $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

APPENDIX 1
DEFINITIONS OF CHROMOSOME ABERRATIONS SCORED IN METAPHASE PORTRAITS

Aberration	Abbreviation	Description
Chromatid gap	g'	An achromatic lesion which appears as an unstained region in the chromatid arm, the size of which is equal to or smaller than the width of the chromatid and the apparently "broken" segments of the chromatid arm are in alignment.
Chromosome gap	g"	An achromatic lesion which appears as an unstained region in both chromatids at the same position, the size of which is equal to or smaller than the width of the chromatid and the apparently "broken" segments of the chromatids are in alignment.
Chromatid break	b'	An achromatic lesion in a chromatid arm, the size of which is larger than the width of the chromatid. The broken segments of the chromatid arm are aligned or unaligned.
Chromosome break	b"	An achromatic lesion in both chromatids at the same position, the size of which is larger than the width of the chromatid. The broken segments of the chromatids are aligned or unaligned.
Chromatid deletion	d'	Deleted material at the end of a chromatid arm.
Minute	m'	A single, usually circular, part of a chromatid lacking a centromere.
Double minutes	m"	Two, usually circular, parts of a chromatid lacking a centromere.
Dicentric chromosome	dic	A chromosome containing two centromeres.
Tricentric chromosome	tric	A chromosome containing three centromeres.

APPENDIX 1 Continued

Aberration	Abbreviation	Description
Ring chromosome	r	A ring structure with a distinct lumen.
Exchange figure	exch.	An exchange(s) between two or more chromosomes resulting in the formation of a tri- or more-armed configuration.
Chromosome intrachange	intra	A chromosome intrachange is scored after rejoining of a lesion within one chromosome.
Pulverized chromosomes	p	A fragmented or pulverized chromosome
Multiple aberrations	ma	A metaphase spread containing ten or more of the above mentioned aberrations (chromatid and chromosome gaps not included).
Polyploidy	poly	A chromosome number that is a multiple of the normal diploid number.
Endoreduplication	endo	A form of polyploidy in which each centromere connects two or four pairs of chromatids instead of the normal one pair.

APPENDIX 2

STATISTICAL EVALUATION OF TEST RESULTS

Chi-square Test

TOTAL NUMBER OF CELLS WITH ABERRATIONS; TREATMENT/CONTROL COMPARISON, (INCLUSIVE/EXCLUSIVE GAPS).

Experiment 1

TREATMENT DOSE (µg/ml)	S9-MIX	GAPS	P-VALUE two-sided	DECISION AT 95% CONFIDENCE LEVEL
24 h fixation period				
3	-	+	=0.0104	significant
		-	=0.0324	significant
10	-	+	=0.0324	significant
		-	=0.1010	not significant
17.8	-	+	=0.0060	significant
		-	=0.0324	significant
MMC-C (0.2)	-	+	≤0.0004	significant
		-	≤0.0004	significant
422	+	+	=0.0022	significant
		-	≤0.0004	significant
CP (15)	+	+	≤0.0004	significant
		-	≤0.0004	significant
48 h fixation period				
33	-	+	≤0.0004	significant
		-	≤0.0004	significant
MMC-C (0.1)	-	+	≤0.0004	significant
		-	≤0.0004	significant

Experiment 2

TREATMENT DOSE (µg/ml)	S9-MIX	GAPS	P-VALUE two-sided	DECISION AT 95% CONFIDENCE LEVEL
24 h fixation period				
17.8	-	+	=0.0036	significant
		-	≤0.0004	significant
33	-	+	≤0.0004	significant
		-	≤0.0004	significant
MMC-C (0.2)	-	+	≤0.0004	significant
		-	≤0.0004	significant
333	+	+	=0.0658	not significant
		-	=0.0060	significant
CP (15)	+	+	≤0.0004	significant
		-	≤0.0004	significant